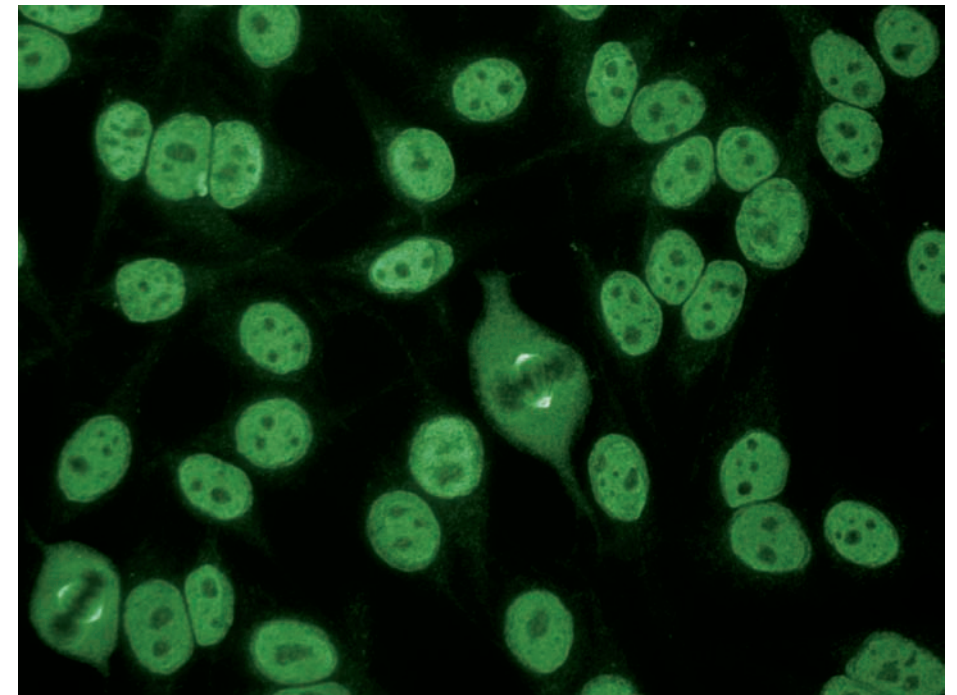

Disease-associated autoantibodies become more and more important for routine diagnostics as well as basic and applied research. As many of these autoantibodies are detectable long time before clinical manifestations, they may be used to predict the development of the appropriate disease. However their potential role in the very early diagnosis or risk assessment of disease development remains to be further studied. The current knowledge, the facts and perspectives regarding the prediction of organ specific and systemic autoimmune diseases are discussed in Chapter 1. For disease prediction and early intervention it is necessary to understand the pathologic processes leading to autoimmune diseases. For instance, components of the innate immune system (e.g. Toll-like receptors) can have a dramatic impact on autoantibody response and disease pathogenesis, either by promoting or by regulating disease (Chapter 2). The different effects of autoantibodies in immune homeostasis and autoimmune manifestations are discussed in Chapters 3 to 6.1 (natural autoantibodies as catalytic or protective immunoglobulins, autoantibodies against protective molecules, macromolecular complexes, receptor structures and ion channels). Reviews and news regarding autoantibodies in organ specific (Chapter 6) and systemic autoimmune diseases (Chapter 7) follow. The main focus of Chapter 7 is the pathologic, diagnostic and prognostic relevance of autoantibodies against citrullinated proteins or peptides. Chapter 9 deals with methodical aspects and diagnostic strategies starting with general comments on early diagnosis of autoimmune rheumatic diseases. Technologies for the identification of novel autoantibodies as well as for the determination of autoantibodies and autoantibody profiles were presented. Improvement of autoantibody analyses by autoantigen designing and technological innovations were discussed. Optimized, standardized and cost-effective multiparametric assays are the prerequisite for a probable future use of autoantibodies for the more accurate prediction of diseases.



K. Conrad, E.K.L. Chan, M.J. Fritzler, U. Sack, Y. Shoenfeld, A.S. Wiik (Eds.)

From Etiopathogenesis to the Prediction of Autoimmune Diseases: Relevance of Autoantibodies

Report on the 8th Dresden Symposium on Autoantibodies held in Dresden on September 12-15, 2007



AUTOANTIGENS, AUTOANTIBODIES, AUTOIMMUNITY
Volume 5 - 2007

AUTOANTIGENS, AUTOANTIBODIES, AUTOIMMUNITY

Edited by: K. Conrad (Dresden, Germany)

U. Sack (Leipzig, Germany)

Vol. 5 — 2007

K. Conrad, E. K. L. Chan, M. J. Fritzler, U. Sack, Y. Shoenfeld,
A. S. Wiik (Eds.)

From Etiopathogenesis to the Prediction of Autoimmune Diseases: Relevance of Autoantibodies

Report on the 8th Dresden Symposium on Autoantibodies held in Dresden
on September 12–15, 2007

AUTOANTIGENS, AUTOANTIBODIES, AUTOIMMUNITY
Volume 5 — 2007



PABST SCIENCE PUBLISHERS
Lengerich, Berlin, Bremen, Miami,
Riga, Viernheim, Wien, Zagreb

Contents

Preface XIX

Chapter 1 Prediction of Autoimmune Diseases — Facts and Perspectives

- 1 Prediction of autoimmunity — more than just autoantibodies 2
Yehuda Shoenfeld
- 2 Predictive autoantibodies: Past, present and future 6
Noel R. Rose
- 3 The predictive relevance of autoantibodies 16
Karsten Conrad, Werner Schössler, Marvin J. Fritzler

Chapter 2 Induction of Autoantibodies

- 4 The role of Toll-like receptors in the induction of autoantibodies and the development of autoimmune diseases 34
Maria Fischer, Alexander Stoehr, Jeffrey V. Ravetch, Marc Ehlers
- 5 Autoimmune responses in experimental models of rheumatoid arthritis 45
Günter Steiner, Markus Hoffmann, Silvia Hayer, Ruth Fritsch, Josef Smolen
- 6 Origin of anti-neutrophil cytoplasmic antibodies (ANCA) — new insights 54
Elena Csernok, Julia Ulrike Holle, Wolfgang Ludwig Gross

Chapter 3 Effects of Autoantigens and Autoantibodies

- 7 Protective autoantibodies: Role in homeostasis, clinical importance and therapeutic potential 62
Elias Toubi, Yehuda Shoenfeld
- 8 Catalytic autoantibodies: Role in immune homeostasis and clinical autoimmunity 77
Mikhail Paltsev, Aleksandr Gabibov, Sergey Suchkov
- 9 Catalytic autoantibodies in organ-specific autoimmunity: an update 127
Elena Tretyak, Sergey Suchkov
- 10 Antiphospholipid antibodies induce procoagulant cytokine activity stimulating the TLR8 pathway 130
Philipp von Landenberg, Yvonne Döring, Maik D. Drechsler, Natascha Clemens, Ingrid Obermeier, Stefan Bauer, Joab Chapman, Yehuda Shoenfeld, Miri Blank, Karl J. Lackner
- 11 Clinical and pathological significance of autoantibodies against protective molecules 131
Martine Szyper Kravitz, Yehuda Shoenfeld
- 12 Are autoantibodies secreted by long-lived plasma cells pathogenic? 141
Falk Hiepe, Thomas Dörner, Rudolf Manz, Andreas Radbruch
- 13 LEDGFp75/DFS70, a stress response autoantigen with multiple functions and broad clinical relevance 146
Melanie Mediavilla-Varela, Lai Sum Leoh, Anamika Basu, Vidya Ganapathy, Carlos A. Casiano

Chapter 4 Autoantibodies to Macromolecular Complexes

- 14 Autoantibodies to key components of the RNA interference pathway 168
Keigo Ikeda, Kaleb M. Pauley, Andrew Jakymiw, Minoru Satoh, Marvin J. Fritzler, Edward K. L. Chan
- 15 Autoantibodies to cytoplasmic autoantigens in endosomes, exosomes and the Golgi Complex 178
Marvin J. Fritzler, Laura M. Stinton, Edward K. L. Chan
- 16 Novel aspects of autoantibodies to the human exosome (PM/ScI complex) 194
Michael Mahler, Marvin J. Fritzler

- 17 The human interferon-inducible protein IFI16: a mediator of inflammation and a target for autoimmunity 210
Pier Luigi Meroni, M. Mondini, Paolo Airò, Piersandro Riboldi, Yehuda Shoenfeld, Marisa Gariglio, Santo Landolfo

Chapter 5 Receptors, Autoantibodies, and Disease

- 18 Autoimmunity and idiopathic dilated cardiomyopathy: Where we are? 216
Michael Fu
- 19 Functional autoantibodies against G-protein coupled receptors 222
Gerd Wallukat

Chapter 6 Autoantibodies in Organ Specific Autoimmune Diseases

6.1 Neuromuscular and Neurological Diseases

- 20 Pathogenic and diagnostic relevance of autoantibodies against ion channels 236
Bethan Lang, Angela Vincent
- 21 Surface-binding autoantibodies and B-cell activating factor (BAFF) in pediatric opsoclonus-myoclonus syndrome 247
Sandra Bick, Stefanie Altenkämper, Isabell Krasenbrink, Marlene Tschernatsch, Antje Kirsten, Franz Blaes, Verena Fühlhuber
- 22 Anti-neuropil antibody in a patient with limbic encephalitis and breast cancer 255
R. Vitaliani, M. Zoccarato, S. Irani, Sergio Zanini, Gian Luigi Gigli, B. Giometto, A. Vincent
- 23 Paraneoplastic limbic encephalitis with anti-Ma2 and CV2 antibodies 257
B. Giometto, M. Zoccarato, L. Zuliani, R. Vitaliani
- 24 Long term survival of two patients with small cell lung carcinoma and anti-Hu antibodies. Relation to therapy and serial antibody determination. 258
U. Wurster, J. Tümmeler, E. Sindern, A. Borchert, M. Schilling
- 25 Possible implementation of diagnostic tools in paraneoplastic neurological disorders 261
D. Saccomanno, G. P. Comi, N. Bresolin, M. Carpo

- 26 EUROLINE neuronal antigens: A newly developed line immunoassay for the detection of antibodies against neuronal antigens in paraneoplastic syndromes 263
T. Scheper, W. Meyer, P. Klatt, L. Komorowski, C. Probst, W. Schlumberger, W. Stöcker
- 27 Complex regional pain syndrome and autoantibodies against differentiation-dependent neuronal surface antigens 265
F. Blaes, D. Nascimento, K. Schmitz, O. Matz, M. Tschernatsch, M. Kaps
- 28 Antineurofilament antibodies in cerebrospinal fluid and serum in patients with amyotrophic lateral sclerosis 267
I. Malbohan, L. Fialová, J. Švarcová, A. Bartoš, P. Ridzoň, R. Rusina
- 29 Avidity of antineurofilament antibodies determined by ELISA method 269
I. Malbohan, J. Švarcová, L. Fialová, A. Bartoš
- 30 Anti-ganglioside autoantibody profiling in patients with autoimmune peripheral neuropathies by a new line immunoassay 271
Hauke Schneider, Karsten Conrad, Ulrich Canzler, Tjalf Ziemssen, Thomas Talaska, Dirk Reinhold, René Louis Humbel, Dirk Roggenbuck
- 31 Detection of anti-MOG autoantibodies in acute disseminated encephalomyelitis (ADEM) by FACS analysis using MOG-transfected LN18 cells 276
P.W. Modderman, L. A. Aarden, R. Q. Hintzen, D. Hamann
- 32 Discovery and validation of novel multiple sclerosis associated biomarkers using protein biochips 278
A. Lueking, C. Gutjahr, K. Schulte, V. Grub, H. E. Meyer, C. Huels, S. Mueller, J. Beator
- 33 Neurological complications in celiac disease: Could have an autoimmune origin? 279
M. Carpo, D. Saccomanno, M. T. Bardella, S. Allaria, N. Bresolin, G. P. Comi
- 34 Spectrum of autoantibodies in patients with psychiatric manifestations 281
L. Laadhar, O. Sidhom, S. Masmoudi, M. Zitouni, M. Sellami-Kallel, H. Zouhei, S. Makni

6.2

Gastrointestinal and Liver Diseases

- 35 Anti-carbohydrate antibodies as new markers for inflammatory bowel disease 284
Karin Malickova, Peter Laszlo Lakatos, Robert Donoval, Petra Sandova, Ivana Janatkova, Milan Lukas

-
- 36 Differential diagnosis between Crohn's disease vs non-CD patients using a combination of antiglycan antibodies (gASCA, ALCA, ACCA and AMCA) 294
Margarida Franco, Mónica Ramalho, Sara Alberto, Luís Novais, Germano de Sousa, João Ramos de Deus
- 37 Aberrant expression of B cell-activating factor of the TNF family (BAFF) and its receptor in duodenal biopsies of patients with celiac disease 297
M. Fabris, F. Barone, A. Lerussi, D. Visentini, A. Picierno, R. Maieron, D. Villalta, S. De Vita, N. Bizzaro, R. Tozzoli, S. Pizzolitto, J. Spencer, E. Tonutti
- 38 Overexpression of B cell-activating factor of the TNF family (BAFF) in serum and duodenal biopsy in celiac disease patients. 299
M. Fabris, A. Lerussi, F. Barone, J. Spencer, D. Visentini, A. Picierno, R. Maieron, D. Villalta, F. Curcio, S. De Vita, N. Bizzaro, R. Tozzoli, E. Tonutti
- 39 Deamidated gliadin peptides are superior to native gliadin in ELISA for diagnosis of childhood celiac disease 302
Christian Prause, Christian Probst, Conny Dähnrich, Wolfgang Schlumberger, Winfried Stöcker, Thomas Richter, Almuth Christine Hauer, Martin Stern, Holm Uhlig, Martin W. Laass, Klaus-Peter Zimmer, Thomas Mothes
- 40 Antibodies against deamidated gliadin peptides as novel biomarkers of childhood celiac disease 308
Maria Ritter, Rüdiger Lieske, Thomas Richter, Almuth Christine Hauer, Martin Stern, Holm Uhlig, Martin W. Laass, Klaus-Peter Zimmer, Thomas Mothes
- 41 Screening for celiac disease in patients with Hashimoto thyroiditis 313
L. Laadhar, F. Harzallah, A. Hassin, M. Zitouni, M. Kallel-Sellami, S. Masmoudi, H. Slimane, S. Makni
- 42 Production of celiac disease autoantibodies after *in vitro* gliadin challenge is dependent on small-bowel mucosal transglutaminase 2-specific IgA deposits 315
S. Stenman, K. Lindfors, Ilma R. Korponay-Szabo, O. Lohi, H. Wieser, Markku Mäki, Katri Kaukinen
- 43 Celiac disease-specific IgA class autoantibodies disturb angiogenesis 317
Essi Myrsky, Katri Kaukinen, Mari Syrjänen, Ilma R. Korponay-Szabo, Markku Mäki, Katri Lindfors
- 44 Detection of primary biliary cirrhosis-associated anti-mitochondrial antibodies using an improved test system: Anti-M2/BPO ELISA 319
L. Komorowski, D. Bogdanos, C. Probst, C. Dähnrich, A. Rosemann, W. Schlumberger, W. Stöcker

- 45 An help on primary biliary cirrhosis diagnostics?
Comparison between a newly developed ELISA (PBC Screen) and the Sp100, gp210
ELISAS 321
T. Martins, I. Abreu, C. Cardoso, A. Bastos, H. Trindade, J. Chaves
- 46 A comprehensive line immunoassay for the detection of autoantibodies in primary
biliary cirrhosis (PBC) 323
*W. Meyer, T. Scheper, N. Janssen, L. Komorowski, C. Probst, W. Schlumberger, D. Bogdanos,
W. Stöcker*

6.3 Endocrine Diseases

- 47 Autoantibody characteristics and combinations in the prediction of type 1 diabetes 326
Ezio Bonifacio, Peter Achenbach
- 48 Differentiation of type 1 diabetes risk by GAD antibody epitope analysis and insulin
antibody affinity in antibody positive children from a general population 338
*Michael Schlosser, Wolfgang Kerner, Peter Achenbach, Christiane S. Hampe, Reinhard
Walther*
- 49 Examination of the diabetes-associated autoantigen GAD65 in serum as a possible
early diagnostic marker of beta cell loss 340
U. Walschus, I. Klötting, R. Walther, M. Schlosser

6.4 Cutaneous Autoimmune Diseases

- 50 Prevalence of autoantibodies in patients with pemphigus 344
K. Mejri, M. Kallel-Sellami, L. Laadhar, H. Lahmer, M. Zitouni, S. Makni
- 51 Successful anti-CD20 therapy in paraneoplastic pemphigus associated with a
follicular dendritic cell sarcoma 346
*Romain Bailloud, Jacques Serratrice, Sophie Desplat-Jégo, Pascal Thomas, Pierre-Jean
Weiller*
- 52 Sensitive and specific detection of pemphigoid autoantibodies by an Enzyme-linked
immunosorbent assay using multimers of the NC16A domain of BP180 as antigen 348
*C. Probst, C. Dähnrich, L. Komorowski, I. M. Bloecker, E. Schmidt, W. Schlumberger, C.
Sitaru, C. Rose, W. Stöcker, D. Zillikens*

Chapter 7 Autoantibodies in Systemic Autoimmune Diseases

- 53 Autoantibodies, classification criteria and diagnosis of systemic autoimmune rheumatic diseases 352
Jennifer G. Walker, Cheryl Barnabe, Marvin J. Fritzler

- 54 Anti-phospholipid antibodies as predictors of autoimmune disease manifestations 370
Yaniv Sherer, Yehuda Shoenfeld

7.1 Rheumatoid Arthritis and Other Arthropathies

- 55 Citrullination in the arthritic synovium; the citrullinome, the antibodies against citrullinated proteins and their connection with RA pathogenesis 378
Joyce J. B. C. van Beers, Albert J. W. Zendman, Walther J. van Venrooij, Ger J. M. Pruijn

- 56 Differential prognostic impact of autoantibodies and inflammation markers in early rheumatoid arthritis 389
Johan Rönnelid, Mohammed Mullazehi, Linda Mathsson

- 57 Clinical and pathophysiological relevance of the autoimmune response to citrullinated proteins 401
Guy Serre

- 58 Should rheumatoid patients have flossed more? Maybe ... 404
H. A. Ménard, M. Lora, William V. Giannobile, J. Mobley, D. Grenier, C. Bodet

- 59 Comparison of anti-mutated citrullinated vimentin antibodies with anti-CCP antibodies: Interest in RA diagnosis and during infliximab therapy 406
P. Nicaise-Roland, S. Grootenboer-Mignot, A. Bruns, M. Hurtado, E. Palazzo, G. Hayem, O. Meyer, S. Chollet-Martin

- 60 Improvement of serological RA diagnostics by autoantibody profiling 408
Karsten Conrad, Babette Heschel, Andrea Thieme, Renate Christoph, Beate Roch, Kirsten Lüthke, Stefan Bornstein, Hans-Egbert Schröder

- 61 Cost effectiveness of autoantibodies against cyclic citrullinated peptide in the very early diagnosis of rheumatoid arthritis 411
Alexander Konnopka, Karsten Conrad Christoph Baerwald, Hans-Helmut König

- 62 Characterization of cells expressing citrullinated proteins in synovial tissue of patients with rheumatoid arthritis, reactive arthritis, psoriatic arthritis and osteoarthritis 413
Christof Zimmermann, Makiyeh Tohidast-Akrad, Peter Zenz, Josef Smolen, Günter Steiner
- 63 Antibodies cross-reacting with a 28 kDa drosophila antigen for diagnosis of ankylosing spondylitis? 415
C. Duftner, C. Dejaco, H. J. Lakomek, M. Schirmer
- 64 Hypothesis-testing for etiopathogenesis, therapy and prevention of ankylosing spondylitis, Klebsiella-reactive uveitis and rheumatoid arthritis 423
Frank Hartig, Roland Pechlaner
- 65 Autoantibody profile in slovak patients with juvenile idiopathic arthritis 425
D. Kozáková, V. Bošák, E. Košková, F. Mateička

7.2

SLE and Antiphospholipid Syndrome

- 66 Antibodies against nucleosomes and DNA: Clinical and pathogenic significance 428
L. Cebeacauer, I. Lochman, V. Král, R. W. Burlingame, W. Schlumberger
- 67 Can anti-chromatin antibody ELISA replace FARR in a routine diagnostic laboratory? 440
Myfanwy Spellerberg, Matthew Hayman, Peter Chapman, Lisa Stamp, John O'Donnell
- 68 Evaluation of novel assay systems for the determination of autoantibodies to double-stranded DNA in patients with SLE 442
Christof Zimmermann, Elisabeth Hoefler, Josef Smolen, Günter Steiner
- 69 A novel dot assay for the detection of anti-dsDNA antibodies in SLE sera 444
Tanja Lüttich, Petra Eißfeller, Christine Jauris, Arno Kromminga
- 70 Characterization of cellular and humoral autoimmune responses to histone H1 and core histones in patients with SLE 446
Georg Stummvoll, Ruth Fritsch, Brigitte Meyer, Martin Aringer, Josef Smolen, Günter Steiner
- 71 Cryopreservation of crithidia luciliae 448
Sonia Carujo, Ana Belén Polonio, Petraki Munujos

- 72 High sensitive detection of double-stranded DNA autoantibodies by a modified Crithidia luciliae immunofluorescence test (CLIFT) 450
Werner Schöblier, Dirk Roggenbuck, Thomas Büttner, Uta Kießling, Hans-Egbert Schröder, Karsten Conrad
- 73 The association of anti-SS-A/Ro52 and anti-SS-A/Ro60 antibodies in different connective tissue diseases 453
Petra Eißfeller, Birgit Pepperkok, Tanja Luettich, Michael Mahler, Christine Jauris, Marvin J Fritzler
- 74 Evaluation of novel chemiluminescence-based methods for the detection of anti-cardiolipin antibodies in APS and SLE patients 463
Alexander W. Götz, Francesco Capuano, Luca Pallavicini, Karsten Conrad, Kristin Tausche, Hans-Egbert Schröder
- 75 Value of serum levels of autoantibodies for monitoring therapy with aphaeresis in antiphospholipid syndrome (APS) 465
M. Tampoia, A. Ramunni, A. Zucano, P. Lisi, G. Pannarale, A. Fontana

7.3 Systemic Sclerosis

- 76 Evaluation of an enzyme immunoassay for the detection of scleroderma-related autoantibodies 468
R. Tozzoli, G Kodermaz, N. Bizzaro, E. Tonutti, M. Tampoia, S. Platzgummer, A. Antico
- 77 RNA polymerase III antibodies in patients with systemic sclerosis: Validation of a new ELISA method 471
R. Tozzoli, G Kodermaz, N. Bizzaro, E. Tonutti, M. Tampoia, S. Platzgummer, A. Antico, G. Morozzi, D. Bassetti
- 78 Anti-Scl70 antibody levels correlate with skin and organ fibrosis in patients with systemic sclerosis — analysis from the Charité SSc cohort 475
Stefanie Uibel, Katharina Hanke, Cornelia Dähnrich, Claudia Brückner, Karl Egerer, Falk Hiepe, Wolfgang Schlumberger, Gabriela Riemekasten
- 79 Antibodies against CENP-B antigen identify a subset of systemic sclerosis patients with sicca syndrome and missing lung fibrosis — analysis of the Charité SSc cohort 477
Katharina Hanke, Stefanie Uibel, Claudia Brückner, Cornelia Dähnrich, Karl Egerer, Falk Hiepe, Wolfgang Schlumberger, Gabriela Riemekasten

7.4**ANCA-associated Vasculitis**

- 80 MPO and PR3 autoantibodies: Evaluation of a new indirect immunofluorescence method 480
F. Gioia, D. De Francesco
- 81 A novel ELISA detects anti-neutrophil cytoplasm antibodies against proteinase 3 with superior sensitivity 482
Bernhard Hellmich, Elena Csernok, Gert Fredenhagen, Wolfgang L. Gross
- 82 EUROPLUS™ ANCA BIOCHIP Mosaic: MPO and PR3 antigen dots improve the detection of ANCA by indirect immunofluorescence 485
J. Damoiseaux, M. Buschtez, U. Steller, B. Zerbe, A. Rosemann, K. Fechner, W. Schlumberger, J. W. Cohen Tervaert, W. Stöcker
- 83 A newly developed ELISA using a mixture of native PR3 and recombinant PR3 expressed in human cells improves the serological investigation of ANCA-associated vasculitis 487
J. Damoiseaux, C. Dähnrich, A. Rosemann, C. Probst, L. Komorowski, W. Schlumberger, E. Csernok, F. Hiepe, J. W. Cohen Tervaert

Chapter 8**Autoantibodies in Miscellaneous Diseases**

- 84 Anti-GSTT1 antibody mediated chronic renal allograft rejection 490
I. Wichmann, I. Aguilera, A. J. Alvarez-Marquez, M. A. Gentil, A. Nuñez-Roldan
- 85 The antinuclear autoantibodies in patients with chronic heart failure 492
N. Virstyuk, E. Cherkachuna
- 86 Determination of serum anti-S100 autoantibodies in patients with acute ischemic stroke 494
M. Cojocar, I. M. Cojocar, C. Burcin, A. Atanasiu
- 87 Prevalence and clinical significance of autoantibodies in 147 adult patients with cystic fibrosis 496
Karine Nkana, Florence Lachenal, Raphaelle Nove Josserand, Isabelle Durieu, Nicole Fabien
- 88 High prevalence of autoantibodies in patients with sickle cell disease 498
A. M. Rouquette, S. Obadia, P. M'Bappe, I. Hagege, F. Boussa-Khettab, L. Tshilolo, R. Girot

Chapter 9 Methodical Aspects and Diagnostic Strategies

- 89 Strategies and algorithms to improve the early diagnosis of autoimmune rheumatic diseases 502
Allan Wiik
- 90 Autoantibodies in individuals with no apparent autoimmune disease 510
Silvia Helena Barbosa, Danilo Mesquita Junior, Paulo Guilherme Leser, Alessandra Dellavance, Luís Eduardo Coelho Andrade
- 91 Definition of reference intervals in autoantibody assays using indirect methods based on current data 530
R. Tozzoli, D. Giavarina, D. Villalta, N. Bizzaro
- 92 Lessons from autoantibody binding avidity 540
Arno Kromminga

9.1 Identification of Novel Autoantibodies

- 93 Systematic development of antibody profiles as biomarkers 544
Angelika Lueking, Axel Kowald, Helmut E. Meyer, Jens Beator
- 94 Identification of two new autoantigens in patients with high titres of antinuclear autoantibodies using protein macro-array technology 551
Cindy Hempp, Friedrich Haag, Friedrich Koch-Nolte, Thorsten Krieger
- 95 Protein arrays as versatile tool for the characterization of antibodies 562
Axel Kowald
- 96 Anti-prostasome autoantibodies in serum of prostate cancer patients 563
Karl Göran Ronquist, Lena Carlsson, Gunnar Ronquist, Anders Larsson
- 97 Autoantibodies in patients with glaucoma 578
Stephanie C. Joachim, Jan Reichelt, Simone Berneiser, Norbert Pfeiffer, Franz H. Grus

9.2 Multiplex Assays for Autoantibody Analyses

- 98 Proteomics on a chip for monitoring autoimmune diseases 592
Angelique M. C. Lokate, J. Bianca Beusink, Richard B. M. Schasfoort, Ger J. M. Pruijn

- 99 Development of a novel multi-parameter platform for use in autoimmune diseases — technical aspects and first results on ANA testing 602
U. Klause, M. Rothfuss, H. Eberl, M. Wanger, A. Nichtl, H.-J. Müller, B. Risse
- 100 Evaluation of ANA-ENA antibodies in sera of patients with Sjögren's syndrome using two multiplexed immunoassays (AtheNA multi-lyte ana system and the FIDISTM connective) and two conventional methods (Ouchterlony double immunodiffusion, immunoblotting) 604
I. Abreu, H. Guimarães, A. Bastos, Vaz Pato, F. Barcelos, G. Sousa, H. Trindade
- 101 BioPlexTM2200 system: Simultaneous detection of anti-dsDNA and anti-nucleosome antibodies in patients with systemic lupus erythematosus 606
Nathalie Bardin, Sophie Desplat-Jégo, Bruno Larida, Marielle Sanmarco
- 102 Comparison between UltraPlexTM barcoded microparticle technology and enzyme-linked immunosorbent assay for the determination of antibodies to dsDNA and extractable nuclear antigens 608
Torsten Witte, Jodie Smith, David Mosedale, Reinhold E. Schmidt
- 103 Evaluation of multiplexed fluorescence microsphere immunoassay for detection of autoantibodies to nuclear antigens 611
A. Kozmar, M. Rudolf, I. Radić, Branko Malenica
- 104 EUROLINE Myositis Profile: A newly developed line immunoassay for the detection of myositis specific autoantibodies 612
W. Meyer, T. Scheper, N. Janssen, S. Torkler, W. Schlumberger, W. Stöcker
- 105 Microblots for autoantibody differentiation 614
Kai Großmann, Ulrich Wagner, Rico Hiemann, Stephan Milius, Elke Kalz, Thomas Büttner, Karsten Conrad, Werner Lehmann

9.3

Improvement of Autoantibody Detection

- 106 Designer antigens as diagnostic targets for (auto)antibody determination 619
Christian Probst, Lars Komorowski, Cornelia Dähnrich, Anke Rosemann, Wolfgang Schlumberger, Klaus-Peter Wandinger, Thomas Mothes, Winfried Stöcker
- 107 HEp-2 cell preparation for automated analysis of autoantibodies 632
J. Michel, R. Hiemann, N. Hilger, K. Kaltschmidt, M. Weigert, U. Sack, U. Anderer

108	Autoantibody screening by indirect immunofluorescence on HEp-2 cells: Comparison of a novel automated image processing with visual examination <i>Rico Hiemann, Nadja Hilger, Karsten Conrad, Dirk Roggenbuck, Joerg Michel, Ursula Anderer, Martin Weigert, Ulrich Sack</i>	634
	Color Figures	636
	Subject Index	665
	Author Index	671

Preface

For nearly five decades, autoantibodies with more or less specificity for disease or disease phenotype are used in routine diagnostics, basic as well as applied research. The applied research on autoantibodies has yielded an increasingly important approach to the diagnosis and management of patients with a variety of autoimmune conditions. The detection of autoantibodies in patient's sera is a key first step in most of the known autoimmune diseases. Although it has been shown that many disease-associated autoantibodies are detectable in preclinical stages, their potential role in the very early diagnosis or risk assessment of disease development is, with the exception of diabetes-associated autoantibodies, not entirely clear. Therefore, the fifth AAA volume has focused on the current knowledge about the predictive value of autoantibodies in organ specific and systemic autoimmune diseases. More research, especially long-term, multicenter, prospective studies, is necessary to evaluate the real value of autoantibodies for the prediction of disease development in very early stages.

Furthermore, all mechanisms and factors leading to the induction and maintenance of autoimmunity, as well as to the manifestation of autoimmune diseases, are important in order to develop novel options and strategies in prevention, early diagnostics, and effective therapy of these autoimmune diseases. In the last few recent years it has become clear that the innate immune system has a more important role in autoimmune processes as suggested in earlier reports. For instance, Toll-like receptors can have a dramatic impact on autoantibody response and disease pathogenesis, either by promoting or by regulating disease. Some components of the innate immune system can be regarded as a bridge between exogenous harmful factors and the development of specific autoimmunity. Autoantibodies, as biomarkers of these interactions, may be helpful in the elucidation of these complex pathogenic processes. Some of these aspects will be discussed in this volume along with examples of protective and pathogenic effects of autoantibodies.

In the future, autoantibodies may be used for a more accurate prediction of diseases and disease state, with the hope that early and effective intervention will be able to terminate ongoing pathologic processes. Autoantibody detection must be optimized, standardized, and ideally be cost effective, but even more important must be chosen from a clinical user perspective. Furthermore, the search

for novel autoantibodies with diagnostic or prognostic relevance must proceed. Emerging new technologies for the identification of novel autoantibodies, as well as for the determination of autoantibodies and autoantibody profiles, will be presented. Hopefully, the data and informations described and discussed here will stimulate novel concepts that will further the search for better prediction, prevention and treatment of autoimmune diseases.

The editors

Karsten Conrad
Edward K. L. Chan
Marvin J. Fritzler
Ulrich Sack
Yehuda Shoenfeld
Alan S. Wiik

Chapter 1

Prediction of Autoimmune Diseases — Facts and Perspectives

Prediction of autoimmunity – more than just autoantibodies

Yehuda Shoenfeld

Head, Department of Internal Medicine B and Center for Autoimmune Diseases, Sheba Medical Center, Tel Hashomer, Incumbent of the Laura Schwarz-Kipp Chair for Research of Autoimmune Diseases, Tel-Aviv University, Israel.

A 53 years old mother with anti-phospholipid syndrome and vitiligo, came to my office for advise regarding her daughter, a beautiful 23 year old physical fitness trainer. A routine examination of the daughter revealed on ANA of 1:160, Lupus anticoagulant (LAC), IgA anti-CL and IgG anti-Tg. The mother asked me for my recommendations. Needless to say that the daughter was asymptomatic and sexually active.

Had I been asked for advice 10 years ago, my advice would be limited to a more follow-up, if anything (claiming that “We treat the patient and not the inflammation of the laboratory” – “Laboratitis”). However, the mother and the daughter sought my advice in 2007, after we had extensively reviewed the issue of prediction of autoimmune diseases [1–7]. Hence my advise entailed:

(1) Extension of the evaluation for autoantibodies to additional ones. (2) HLA analysis. (3) To avoid oral contraceptives. (4) If pregnant notify your physician (preferentially in a high risk pregnancy clinic) of being an asymptomatic auto-antibody carrier. (5) Avoid UV. (6) Avoid un-necessary vaccines [8–15]. (7) Keep a diet with enriched unsaturated fatty acid [16]. (8) Avoid smoking [17, 18]. (9) Continue with physical activities. (10) Add Vitamin D 400–800 IU a day to your diet [19].

Four months later, the young lady who was dating a nice fellow, had contracted from him infectious mononucleosis. Following this infection she developed thrombo-phlebitis and pulmonary embolus. Thus establishing a full blown clinical picture of anti-phospholipid syndrome (APS).

This clinical description exemplifies the importance of genetic background in autoimmunity (a mother with APS and vitiligo), the fortuitous detection of autoantibodies in an asymptomatic person, the impact of the triggering environmental factor (EBV infection) in the process of transforming the asymptomatic state of carrying autoantibodies to that of a well defined symptomatic (overt) autoimmune condition.

Previously, we have referred to the combined factors of the “mosaic of autoimmunity” [20, 21] as aiding in our ability to predict eventual involvement of a mere presence of a specific autoantibody (or a combination of several autoantibodies) to an overt autoimmune condition. The “risk factors” entail:

(1) The many autoimmune genetic aspects from specific MHC marker to “notorious” haplotypes (i. e. HLA, A₁, B₈, DR₃), (2) TNF α , TNF β , (3) C₄, C₁, C₂, (4) IgG receptors such as F_c γ RIIA, F_c γ RIIIA, (5) Manose binding lectin (MBL), (6) PTPN 22, (7) Osteopontin, (8) TYK₂, (9) IFR5, (10) CTLA-4, (11) CR₁, (12) Il-10, (13) FCRL3.

The hormonal factors include the sex hormones (leading to the higher prevalence of the disease in females) such as estrogens, but also prolactin [22–26]. Among the hormones one should also list the low levels of Vitamin D in all classical autoimmune conditions [19].

The immune system defects may encompass an immune deficiency state such as IgA deficiency, complement deficiencies (C1q, C4, C2), as well as aberrant reaction of the innate immunity (Toll like receptors).

Almost all classical autoantibodies were reported to precede the respective autoimmune disease by months to years [1–3]. Yet, the availability of the multiplex techniques [27, 28] enabling to measure several autoantibodies simultaneously, and the novel emerging protein and glycans cheap arrays — which will lead to the detection of hundreds of autoantibodies, the function of many of them still being obscure today — will lead to a decade of learning of new predicting autoantibodies.

Sophisticated algorithms and the employment of highly computerized formulas may lead by the end of the decade to a considerably improved ability of predicting the future development of autoimmune diseases, in more accurate manner. This ability to predict will be expanded not only to the type of the disease but most probably also to the tissues or organ(s) involved. The lag time of the disease development could be estimated more accurately, although exact time when environmental factors will intervene would remain relatively unknown, with the exception of the post-partum period.

The next decade may also expand our knowledge on the most important aspect of this issue: i.e. preventive measures. Currently our options are limited, avoidance of UV, stress, smoking, some vaccines, and oral contraceptives. We know from extensive experimental models and from limited diverse human studies, that Vitamin D may help to prevent autoimmunity. Yet we do not know the optimal doses (i.e. 400 IU or 2000 IU?). To determine those we will need to perform extensive epidemiological studies.

A more interesting question is whether one of the future biologics such as anti-Blyss, IVIG or one of the anti-cytokines will be valuable in reverting the course from autoimmunity to autoimmune disease.

The next decade will undoubtedly be exciting in all the above aspects, and will lead to better detection, prediction and hopefully better prevention of autoimmune diseases.

References

- [1] Shepshelovich D, Shoenfeld Y. Prediction and prevention of autoimmune diseases: additional aspects of the mosaic of autoimmunity. *Lupus* 2006; 13: 183–190.
- [2] Harel M, Shoenfeld Y. Predicting and preventing autoimmunity, myth of relity? *Ann NY Acad Sci* 2006; 1069: 322–345.
- [3] Bizzaro N, Tozzoli R, Shoenfeld Y. Are we at a stage to predict autoimmune rheumatic diseases? *Arthritis & Rheum* 2007; 56: 1736–1744.
- [4] Shoenfeld Y. Primary biliary cirrhosis and autoimmune rheumatic disease: prediction and prevention. *Isr J Med Sci* 1992; 28: 113–116.
- [5] Shoenfeld Y. What is going to happen as for the prediction of autoimmune disorders is concerned. *Ann Med Intern* 1992; 143: 525–526.
- [6] George J, Ahmed A, Patnaik M, Adler Y, Levy Y, Harats D, Gilbud B, Terrybery J, Shen G-Q, Sagie A, Herz Im Snow P, Brandt J, Peter J, Shoenfeld Y. The prediction of coronary atherosclerosis employing artificial neural networks. *Clin Cardiol* 2000; 23: 453–456.
- [7] Israeli E, Grotto I, Gilburd B, Balicer RD, Wiik A, Shoenfeld Y. Anti-saccharomyces cerevisiae and antineutrophil cytoplasmic antibodies as predictors of inflammatory bowel disease. *Gut* 2005; 54: 1232–1236.
- [8] Cohen AD, Shoenfeld Y. Vaccine-induced Autoimmunity. *J Autoimmunity* 1996; 9: 699–703.
- [9] Shoenfeld Y, Aron-Maor A. Vaccination and autoimmunity — ‘Vaccinosis’: a dangerous liaison? *J Autoimmunity* 2000; 14: 1–10.
- [10] Aharon-Maor A, Shoenfeld Y. The good, the bad and the ugly of vaccination. *IMAJ* 2000; 2: 225–227.
- [11] Shoenfeld Y, Aharon-Maor A, Sherer Y. Vaccination as an additional player in the mosaic of autoimmunity. *Clin Exp Rheumatol* 2000; 18: 181–184.
- [12] Arom-Maor A, Shoenfeld Y. Vaccination and systemic lupus erythematosus: the bidirectional dilemmas. *Lupus* 2001; 10: 237–240.
- [13] Molina V, Shoenfeld Y. Infection, vaccines and other environmental triggers of autoimmunity. *Autoimmunity* 2005; 38: 235–245.

- [14] Tishler M, Shoenfeld Y. Vaccines and autoimmunity. In: *The Autoimmune diseases* 4th Ed. Noel R. Rose & Ian R. Mackay (Editors). Elsevier Publ. Amsterdam, The Netherlands, 2006; pp 309–316.
- [15] Orbach H, Shoenfeld Y. Vaccination infection and autoimmunity: Myth and reality VI-AMR 2005-10-26-28, Beau-Rivage Palace Hotel, Lausanne, Switzerland. *Autoimmun Rev* 2007; 6: 261–266.
- [16] Reifen R, Blank M, Afek A, Kopilowiz Y, Sklan D, Gershwin ME, German B, Yoshida S, Shoenfeld Y. Dietary polyunsaturated fatty acids decrease anti-dsDNA and anti-cardiolipin antibodies production in idiotype induced mouse model of systemic lupus erythematosus. *Lupus* 1998; 7: 192–197.
- [17] George J, Levy Y, Shoenfeld Y. Smoking and immunity. An additional player in the mosaic of autoimmunity. *Scand J Immunol* 1997; 45: 1–6.
- [18] Gerli R, Sherer Y, Vaudo G, Schillaci G, Gilburd B, Giordano A, Bartoloni Bocci E, Aliegrucchi R, Marchesi S, Mannarino E, Shoenfeld Y. Early atherosclerosis in rheumatoid arthritis. Effects of smoking on thickness of the carotid artery intima media. *Ann NY Acad Sci* 2005; 1051: 281–290.
- [19] Aronson Y, Amital H, Shoenfeld Y. Vitamin D and autoimmunity: etiological and therapeutical considerations. *Ann Rheum Dis* 2007; In Press.
- [20] Shoenfeld Y, Isenberg DA. The mosaic of autoimmunity. *Immunology Today* 1989; 10:123–126.
- [21] Brickman CM, Shoenfeld Y. The mosaic of autoimmunity. *Scand J Clin Lab Invest* 2001; 61: 3–15.
- [22] Buskila D, Berezin M, Gur H, Lin HC, Alosachie I, Terryberry JW, Barka N, Shen B, Peter JB, Shoenfeld Y. Autoantibody profile in the sera of women with hyperprolactinemia. *J Autoimmunity* 1995; 8: 415–424.
- [23] Buskila D, Shoenfeld Y. Prolactin, bromocriptine and autoimmune diseases. *Isr J Med Sci* 1996; 32: 23–27.
- [24] Buskila D, Lorber M, Neumann L, Flusser D, Shoenfeld Y. No correlation between prolactin levels and clinical activity in patients with systemic lupus erythematosus (SLE). *J Rheumatol* 1996; 23: 629–632.
- [25] Ahmed AEE, Peter J B, Shoenfeld Y. Autoantibodies in angioneurotic edema. *Clin Rev Allergy & Immunol* 1998; 16: 207–210.
- [26] Anaya JM, Shoenfeld Y. Multiple autoimmune disease in a patient with hyperprolactinemia. *IMAJ* 2005; 7: 740–741.
- [27] Shovman O, Gilburd B, Zandman-Goddard G, Yehiely A, Langevitz P, Shoenfeld Y. Multiplexed athena multi-lyte immunoassay for ANA screening in autoimmune diseases. *Autoimmunity* 2005; 38: 105–9.
- [28] Shovman O, Gilburd B, Barzilai O, Shinar E, Larida B, Zandaman-Goddard G, Binder SR, Shoenfeld Y. Evaluation of the BioPlexTM 2200 ANA screen: analysis of 510 healthy subjects: incidence of natural predictive autoantibodies. *Ann NY Acad Sci* 2005; 1050: 280–388.

Predictive autoantibodies: Past, present and future

Noel R. Rose

Departments of Pathology and of Molecular and Microbiology and Immunology
Center for Autoimmune Disease Research
The Johns Hopkins University, Baltimore, Maryland, USA

Key Words

predictive autoantibodies, thyroiditis, Graves' disease, type 1 diabetes mellitus, lupus, genetic markers, susceptibility genes, HLA

Introduction

Recent years have witnessed the introduction of many predictive biomarkers. They are defined as measurable characteristics that can assess normal function, pathologic changes or therapeutic responses predictively. Predictive markers are being applied to autoimmune diseases in a number of different areas including identification of susceptible individuals and populations, forecasting the outcome of clinical trials, aiding in developing new treatments and in the initiation of early or preventative interventions.

In the realm of autoimmune disease the most useful biomarkers to date have been autoantibodies. They are of special value because of their sensitivity and specificity. These terms are defined somewhat differently in a clinical or statistical context. Sensitivity is defined clinically as the proportion of subjects with a particular disease who have a positive test for that disease. In the instance of autoimmune disease, it is the number of subjects with a particular autoantibody

who have the disease divided by the total number of subjects who have the disease [1]. Specificity is the proportion of subjects without the disease who have a negative test result divided by the total number of subjects examined who do not have the disease. In many situations high sensitivity is accompanied by a high rate of so-called false positive reactions which represents a low specificity. Conversely high specificity may involve missing individuals at an early stage of disease or at a late timepoint when antibodies are low or simply missing persons who have poor antibody production. Applying these measures to predictive autoantibodies entails several problems. The first is that sensitivity and specificity may be defined by subjects who will develop the disease in the future rather than merely those who have the disease at the time of measurement. Obviously this figure is usually not known with any degree of certainty. Second, a single antibody may not be sufficient to define an autoimmune disease. Third, in rare diseases, even a small loss in specificity can make an antibody marker misleading because it will identify a relatively large number of individuals who do not have the disease in question, thereby giving a large number of false positives. Thus, the more compelling metric for predictive antibodies takes into account the prevalence of the disease [2]. The predictive value (PV) of positive result is given by the following formula:

$$PV = \frac{\text{true positives}}{\text{true positives} + \text{false positives}}$$

The predictive value of a negative result is:

$$PV = \frac{\text{true negatives}}{\text{true negatives} + \text{false negatives}}$$

Since most autoimmune diseases are rare, the positive predictive value; that is, the possibility of the disease when a particular test is positive, can be quite low. The usefulness of the positive predictive value is, therefore, dependent on the population selected. For example, if a test is applied to the general population, the prevalence of a particular autoimmune disease is generally low and, therefore, the positive predictive value also low. On the other hand, in a population made up of the family of patients, the prevalence can be many fold higher and the positive predictive value of an antibody consequently much greater. For that reason our earlier studies on predictive antibodies were carried out using the parents and siblings of children with autoimmune disease.

The past

Our first major studies on the application of predictive antibodies were carried out at Wayne State University in collaboration with my colleagues, Dr. Lynne Burek and Dr. William Hoffman [3–5]. Recruiting from a large pediatric endocrinology clinic, we were able to study 38 juvenile or adolescent patients with one of the

autoimmune thyroid diseases, chronic lymphocytic thyroiditis or Graves' disease, together with their siblings and parents. We soon found that family background was the strongest predictive marker in these cases of early onset thyroid disease. If both parents had primary evidence of thyroid autoimmunity in the form of thyroid autoantibodies (antibodies to thyroglobulin or to thyroperoxidase), there was a 75 % chance of one of the initially euthyroid siblings of our patient having thyroid antibodies. An euthyroid child in a family with only one parent with thyroid autoimmunity had a 54 % chance of developing autoantibodies; while in families in which neither parent showed evidence of thyroid autoantibodies, the initially normal child had only a 29 % chance of developing thyroid autoantibodies. This rate of antibody positivity was already much higher than found in children in which no autoimmune thyroid disease has been discovered in the family.

Since environmental factors are probably equally distributed among the three groups, we can attribute these differences in the prevalence of thyroid autoimmunity to heredity. In fact, two genetic markers of susceptibility had already been discovered at that time — the HLA haplotype and Gm allotype. We took advantage of our families to search for additional genetic traits that may raise the threshold of susceptibility to autoimmune thyroid disease or related autoimmune disorders. In those days before the availability of the complete human genome, we had to rely on individual genetic markers. In families of a child with autoimmune thyroid disease, we found associations with a number of well established genetic traits including ABO, Rh and Duffy blood groups (in addition to confirming the already known HLA and Gm associations). These studies as well as many others performed since have emphasized that most autoimmune diseases are related to the accretion of a number of genetic traits that raise (or sometimes decrease) the likelihood of occurrence of an autoimmune disease. An exciting recent finding is that a number of these genes, such as those determining CTLA4 and PTPN22, influence susceptibility to several autoimmune disorders [6]. The finding predicts that many of the traits adding to the "autoimmune diathesis" represent key genes regulating the immune response.

The role of HLA in the families of children with chronic lymphocytic thyroiditis was strikingly different from its role in the Graves' disease families. Susceptibility to Graves' thyrotoxicosis is clearly associated with HLA-B8 suggesting that this haplotype favors presentation of an immunodominant epitope on the thyrotropin receptor. In contrast, no single HLA haplotype predominated in the pediatric population susceptible to thyroiditis. However, 89 % of the siblings who shared both haplotypes with the proband had evidence of thyroid autoimmunity. Of the haplotype of the siblings who shared only one haplotype with the proband, 69 % had thyroid antibodies. In siblings sharing no haplotype with a patient, only 56 % had thyroid antibodies. Moreover, when they were examined in the clinic during later follow-up, 32 % of the siblings who had a two haplotype match with the proband had some biochemical or clinical evidence of thyroid dysfunction. If

only one haplotype was shared, one of ten siblings had subclinical thyroid disease, whereas in siblings sharing no haplotype with a patient, none had any evidence of thyroid dysfunction. Thus HLA is an important determinant of susceptibility to thyroiditis, but in the instance of thyroiditis the particular HLA haplotype conferring susceptibility differs from family to family. The findings suggest that different individual families respond to different antigenic determinants on the large thyroglobulin molecule.

Another important biomarker of susceptibility that emerged in our study was race or ethnicity. Of 18 patients with Graves' disease, 6 self identified as white and 12 as black. In contrast, among 20 thyroiditis patients, 18 were white and only 2 were black. Thus, among African American children with thyroid disease, most (75 %) were diagnosed with Graves' disease rather than thyroiditis, whereas among Caucasian children with an autoimmune thyroid disease, most (85 %) have thyroiditis.

Age of onset and sex are also predictive biomarkers. In prepubertal children the rate of males to females was nearly equal. After puberty two thirds of the patients with autoimmune thyroid disease were female.

Finally, thyroid autoantibodies themselves proved to be the most useful biomarker of susceptibility to present or future disease. Thyroid autoantibodies were found in all of the probands and in 50 % of the siblings who were euthyroid at the time of initial examination. Almost all cases of thyroiditis (75 %) or of Graves' disease (89 %) had antibodies to both thyroglobulin and thyroperoxidase; only a few individuals had antibodies to thyroglobulin or thyroperoxidase alone. In the siblings of a pediatric patient with autoimmune thyroid disease, the finding of both antibodies signified a greater risk of subclinical or impending disease than if no antibody or either antibody alone were detected.

In further studies, we were able to gain some information about the precise specificity of antibodies to thyroglobulin [7]. Using a large panel of monoclonal antibodies, Herbert Bressler in our team found that we could distinguish two general types of antigenic determinants on the thyroglobulin molecule. One group of determinants included thyroxine and was broadly cross-reactive among different mammalian species, whereas the other was fairly specific for human thyroglobulin. With Patrizio Caturegli [8] we were able to show that the antibodies present in many euthyroid individuals reacted with the evolutionarily conserved, broadly shared determinants on thyroglobulin, whereas sera from patients with thyroiditis or Graves' disease also recognized the clusters of epitopes that were predominantly human-specific. The shared determinants are probably the most primitive and relate to the core function of the thyroid-hormone whereas species-limited determinants are probably newer in an evolutionary sense.

Putting together the fruits of our own early investigation plus studies carried out since that time, one can assemble an instruction list of predictive biomarkers of autoimmune thyroid disease. They include: 1. family history; 2. age of onset

and sex; 3. presence of multiple thyroid-specific autoantibodies; 4. particular HLA haplotype; 5. non-HLA immunoregulatory genes; 6. ethnicity or race; and 7. production of autoantibodies to disease-associated epitopes.

The present

Our early studies on autoimmune thyroid disease serve as a model for investigating predictive biomarkers for a number of other autoimmune diseases. At the present time, the most advanced work relates to type 1 diabetes mellitus (T1DM) [9, 10]. The disease lends itself to this type of study because of its prolonged natural history and gradual progression. Individuals who are genetically susceptible to the disease develop autoantibodies relatively early, signifying the initiation of insulinitis and beta cell injury. As the loss of beta cell mass continues, metabolic abnormalities begin due to a loss in insulin production and ultimately sufficient beta cell death produces overt hyperglycemia and diabetes.

Although most individuals with type 1 diabetes do not have a family history, the risk of disease in a first degree relative of a T1DM patient is approximately 15 % greater than the general population. The HLA haplotypes associated with the greatest risk are HLA-DR3 and HLA-DQ2, whereas HLA-DR2 is reported to be protective. In addition to the insulin gene, CTLA4 and PTPN22 have been associated with the risk of developing T1DM as well as autoimmune thyroid disease.

Autoantibodies represent the major predictive biomarkers of later T1DM in children. Studies carried out in the United States and Europe have shown that autoantibody production can begin as early as the first year of life and may foreshadow the development of diabetes as long as 15 years later. The progression to diabetes, moreover, is related to the number of autoantibodies produced. If antibodies are found to GAD, IA-2 and insulin, approximately 90 % of children will have overt diabetes after seven years of follow-up. If antibodies are produced to two of the antigens, approximately 75 % of children will be diabetic by the age of ten. If antibody is produced to only one of the three antigens, only about 25 % of the children will become diabetic during the 15 year follow-up.

The presence of T1DM is itself a risk factor for development of other endocrine autoimmune diseases. For example, autoimmune thyroid disease occurs in approximately 28 % of T1DM patients compared with approximately 5–6 % in the general population. Moreover, the development of thyroid disease in the diabetic population is predicted by the production of thyroid-specific autoantibodies, since individuals who produce antibodies to thyroperoxidase have about an 80 % probability of developing hypothyroidism compared with only about 10 % of the TPO negative population [11, 12].

Although Addison's disease is rare in the general population, it can be found in 14 to 21 % of patients with T1DM. The production of antibodies to the cytochrome

P450 enzyme 21 hydroxylase serves as an early biomarker for the development of adrenal insufficiency.

A second disease that has been well studied from the view point of predictive biomarkers is systemic lupus erythematosus (SLE). The genetic risk of SLE is conferred by HLA class II alleles DR2 or DR3. In addition, complement factor C4 deficiency increases the frequency of disease. Recent studies have shown that some lupus related autoantibodies precede the clinical manifestations of SLE by many years [13–15]. The autoantibodies with high predictive value include anti-Ro, anti-La, anti-nuclear antibodies, and antiphospholipid antibodies. Anti-double stranded DNA is intermediate in its predictive value, whereas anti-Sm and anti-nRNP are almost coincident with the first clinical evidence of lupus and typically appear within the year of clinical diagnosis. Interestingly the accumulation of autoantibodies seems to reach a plateau at about the time of diagnosis in most patients.

In the subset of patients who develop anti-Ro antibody, available data show that a particular octapeptide is consistently recognized. The peptide is closely related to a sequence found in Epstein-Barr virus nuclear antigen 1 and suggests that a cross reaction with this viral antigen may be an initiating factor in some cases of SLE [16, 17].

The list of other autoimmune diseases for which predictive autoantibodies have been investigated is growing rapidly [18–20]. They include, for example, rheumatoid arthritis (cyclic citrullinated peptide and rheumatoid factor), myositis, (tRNA synthetases), systemic sclerosis (topoisomerase), CREST syndrome (centromere proteins). In celiac disease studies have focused on tissue transglutaminase antibodies; in myasthenia gravis antibodies to the acetylcholine receptor. In pemphigus, desmoglein 3 has been studied as a prediction marker; in primary biliary cirrhosis E2 pyruvate dehydrogenase complex, and in vitiligo, tyrosinase. Yet, contrasting with earlier reports, in multiple sclerosis myelin basic protein and myelin oligodendritic glycoprotein antibodies are not always associated with progression to disease [21, 22]. In the relatives of patients with dilated cardiomyopathy, the presence of cardiac-specific antibodies has been reported to identify relatives at risk of progressing to heart failure [23]. In pregnancy antibodies to thyroperoxidase may be predictive of postpartum autoimmune thyroid disease and diabetes-associated antibodies predictive of gestational diabetes.

The future

Predictive autoantibodies will have multiple uses in future years. First, they may be valuable adjuncts in predicting the likelihood of developing clinical disease before the diagnostic signs are evident. They will have value in teaching us about the natural history of disease, particularly in providing information about the length of the prodromal period when the immune-mediated destructive process

is silently underway. They will also help in better classification of disease. For example, the presence of the characteristic diabetes antibodies in adult patients with diabetes may indicate late onset autoimmune diabetes. Predictive autoantibodies may provide useful information about the prognosis and future course of disease including the nature and severity of complications, and thereby help to design treatment. They may foretell the impending onset of a second or third autoimmune disease in patients with one autoimmune disorder. Finally, predictive autoantibodies may have value in selecting subjects for therapeutic trials in which the likelihood of disease is much greater than in the general population [24].

Another potential use of predictive antibodies is a more individualized approach to therapy using genetic, immunologic and biochemical markers to guide individual care.

In future years, broader application of predictive antibodies will be possible because of advances in proteomics and technologic improvement [25]. These newer developments allow testing a large number of autoantibodies (“multiplexing”) in a relatively rapid and inexpensive manner. Individual profiles of antibody patterns may be predictive, improve diagnostic accuracy and serve as a guide to treatment. In addition, highly purified peptide subunits of autoantigens can greatly increase sensitivity without a loss of specificity [26, 27].

These potential applications have led to a renewed enthusiasm for the discovery and finer characterization of autoantibodies. There are, however, a number of cautions that must be carefully considered. With regard to prediction of future disease, this information is of value if appropriate interventions are possible [28]. In some cases, institution of early treatment can avoid many of the most devastating manifestations of the disease process. There is some indication that early treatment of rheumatoid arthritis can prevent the most crippling effects of the disease [29]. On the other hand, a number of clinical trials have been undertaken to determine if early treatment is useful in T1DM. So far these studies have not given clear-cut benefit [30]. To be acceptable, an early treatment must not only be able to arrest the autoimmune process and prevent the onset of clinical disease, but also be sufficiently safe and free of side effects to use in otherwise healthy individuals. As the use of predictive antibodies increases, the demands for devising safe and effective early interventions will rise.

An alternative approach to early treatment is to identify the environmental trigger factor of an autoimmune disease. If the patient can in some way be separated from the environmental agent responsible, the disease itself may never occur or not reoccur. For example, patients with celiac disease can be treated successfully by placing them on a gluten-free diet even though the inherited susceptibility to the disease is unaltered. Similarly, perennial treatment with antibiotics has prevented progression of rheumatic heart disease in many young people even though they retain their innate susceptibility to rheumatic fever.

Despite the concerns raised above research on predictive biomarkers will certainly accelerate in coming years, taking advantage of the accumulation of genetic, immunologic and biochemical knowledge. In their application to autoimmune disease they promise the greatest good of all — the possibility of preventing the irreversible destructive effects of the autoimmune process. In the past our approach to the treatment of autoimmune disease has too often been confined to remedying damage that has already occurred. The possibility that we can actively intervene to prevent irreversible damage is a goal worth striving for [31].

Summary

The predictive value of autoantibodies has been limited in the past by the relatively low prevalence of most of the autoimmune diseases. If studied within families, autoantibodies have been shown to be useful in predicting impending disease in thyroiditis, Graves' disease, type 1 diabetes mellitus and cardiomyopathy, among others. In a large populational study, autoantibodies have been demonstrated in lupus several years before the onset of clinical disease. The development of rapid through-put, multiple testing promises to open new opportunities for predictive use of autoantibodies. At the same time, the application of predictive approaches to disease raises serious policy and ethical concerns.

References

- [1] Keren DF, Hrusczh, VA. Clinical significance and cost-effectiveness of clinical immunology testing. In: NR Rose, E. Conway de Macario, JD Folds, HC Lane, RM Nakamura, editors. *Manual of Clinical Laboratory Immunology*, 5th edition, ASM Press, Washington, DC, 1997
- [2] Krischer J. The biostatistics of prediction. *Autoimmunity*. 2004; 37:261–3.
- [3] Burek CL, Hoffman WH, Rose NR. The presence of thyroid autoantibodies in children and adolescents with autoimmune thyroid disease and in their siblings and parents. *Clin Immunol Immunopathol*. 1982; 25:395–404.
- [4] Burek CL, Rose NR, Najjar GM, Gimelfarb A, Zmijewski CM, Polesky HF, et al. Autoimmune thyroid disease. In: Panayi GS and David, CS, editors. *Immunogenetics*. Butterworths; 1984. p. 207–233.
- [5] Rose NR, Burek CL. The interaction of basic science and population-based research: autoimmune thyroiditis as a case history. *Am J Epidemiol*. 1991; 134:1073–8.
- [6] Heward JM, Brand OJ, Barrett JC, Carr-Smith JD, Franklyn JA, Gough SC. Association of PTPN22 haplotypes with Graves' disease. *J Clin Endocrinol Metab*. 2007; 92:685–90.
- [7] Rose NR, Bresler HS, Burek CL, Gleason SL, Kuppers RC. Mapping the autoepitopes of thyroglobulin. *Isr J Med Sci*. 1990; 26:666–72.
- [8] Caturegli P, Mariotti S, Kuppers RC, Burek CL, Pinchera A, Rose NR. Epitopes on thyroglobulin: a study of patients with thyroid disease. *Autoimmunity*. 1994; 18:41–9.

- [9] Barker JM, Barriga KJ, Yu L, Miao D, Erlich HA, Norris JM, et al. Prediction of autoantibody positivity and progression to type 1 diabetes: Diabetes Autoimmunity Study in the Young (DAISY). *J Clin Endocrinol Metab.* 2004; 89:3896–902.
- [10] Mrena S, Virtanen SM, Laippala P, Kulmala P, Hannila ML, Akerblom HK, et al. Models for predicting type 1 diabetes in siblings of affected children. *Diabetes Care.* 2006; 29:662–7.
- [11] Burek CL, Rose NR, Guire KE, Hoffman WH. Thyroid autoantibodies in black and in white children and adolescents with type 1 diabetes mellitus and their first degree relatives. *Autoimmunity.* 1990; 7:157–67.
- [12] Park H, Yu L, Kim T, Cho B, Kang J, Park Y. Antigenic determinants to GAD autoantibodies in patients with type 1 diabetes with and without autoimmune thyroid disease. *Ann N Y Acad Sci.* 2006; 1079:213–9.
- [13] Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N Engl J Med.* 2003; 349:1526–33.
- [14] Reveille JD. Predictive value of autoantibodies for activity of systemic lupus erythematosus. *Lupus.* 2004; 13:290–7.
- [15] Heinlen LD, McClain MT, Merrill J, Akbarali YW, Edgerton CC, Harley JB, et al. Clinical criteria for systemic lupus erythematosus precede diagnosis, and associated autoantibodies are present before clinical symptoms. *Arthritis Rheum.* 2007 ; 56:2344–51.
- [16] James JA, Harley JB, Scofield RH. Epstein-Barr virus and systemic lupus erythematosus. *Curr Opin Rheumatol.* 2006; 18:462–7.
- [17] McClain MT, Heinlen LD, Dennis GJ, Roebuck J, Harley JB, James JA. Early events in lupus humoral autoimmunity suggest initiation through molecular mimicry. *Nat Med.* 2005; 11:85–9.
- [18] Bizzaro N. Autoantibodies as predictors of disease: The clinical and experimental evidence. *Autoimmun Rev.* 2007; 6:325–33.
- [19] Bizzaro N, Tozzoli R, Shoenfeld Y. Are we at a stage to predict autoimmune rheumatic diseases? *Arthritis Rheum.* 2007; 56:1736–44.
- [20] Scofield RH. Autoantibodies as predictors of disease. *Lancet.* 2004; 363:1544–6.
- [21] Kuhle J, Pohl C, Mehling M, Edan G, Freedman MS, Hartung HP, et al. Lack of association between antimyelin antibodies and progression to multiple sclerosis. *N Engl J Med.* 2007; 356:371–8.
- [22] Lalive PH, Menge T, Delarasse C, Della Gaspera B, Pham-Dinh D, Villoslada P, et al. Antibodies to native myelin oligodendrocyte glycoprotein are serologic markers of early inflammation in multiple sclerosis. *Proc Natl Acad Sci U S A.* 2006; 103:2280–5.
- [23] Caforio AL, Mahon NG, Baig MK, Tona F, Murphy RT, Elliott PM, et al. Prospective familial assessment in dilated cardiomyopathy: cardiac autoantibodies predict disease development in asymptomatic relatives. *Circulation.* 2007; 115:76–83.
- [24] Notkins AL. New predictors of disease. *Sci Am* 2007; 296:72–9.
- [25] Tozzoli R. Recent advances in diagnostic technologies and their impact in autoimmune diseases. *Autoimmun Rev.* 2007; 6:334–40.
- [26] Mahler M, Mierau R, Bluthner M. Fine-specificity of the anti-CENP-A B-cell autoimmune response. *J Mol Med.* 2000; 78:460–7.

-
- [27] Sharp V, Utz PJ. Technology insight: can autoantibody profiling improve clinical practice? *Nat Clin Pract Rheumatol.* 2007; 3:96–103.
- [28] Hill ID. Serologic testing for celiac disease: primum non nocere! *J Pediatr.* 2007; 150:453–4.
- [29] van der Helm-van Mil AH, le Cessie S, van Dongen H, Breedveld FC, Toes RE, Huizinga TW. A prediction rule for disease outcome in patients with recent-onset undifferentiated arthritis: how to guide individual treatment decisions. *Arthritis Rheum.* 2007; 56:433–40.
- [30] Haller MJ, Gottlieb PA, Schatz DA. Type 1 diabetes intervention trials 2007: where are we and where are we going? *Diabetes and the endocrine pancreas II. Current Opinion in Endocrinology, Diabetes & Obesity.* 2007; 14:283–287.
- [31] Rose NR. Moving from prediction to prevention. *The Scientist.* 2007; Supl.:76–78.

Acknowledgment

The author's research is supported in part by NIH grants HL077611, HL067290 and HL070729.